Hydrolysis of Proteins for Amino Acid Analysis. II. Acid Hydrolysis in Sealed Tubes of Mixtures of ß-Lactoglobulin and Starch

Experiments with a mixture of β-lactoglobulin and starch, simulating the composition of carbohydrate-rich foods and feeds, have been carried out to determine conditions for acid hydrolysis that will permit maximal recovery of amino acids in hydrolysates of such materials. When a large excess of 6*N* HCl is used for hydrolysis, good recoveries of most amino acids are obtained. However, about one-quarter of the tyrosine present is destroyed under the conditions investigated. Some destruction of methionine and cystine may also be attributed to the presence of carbohydrate, but a special method for the determination of these amino acids is available.

A previous report (1) described the problem of preparing acid hydrolysates of foods and feeds, which represent as accurately as possible the amino acid composition of the original material, and outlined an experimental approach to its possible solution. In brief, it was planned to hydrolyze under different conditions highly purified β-lactoglobulin AB, the amino acid composition of which is well-known (2), in the presence of 20 times as much starch and to select those conditions that brought about the least destruction of labile amino acids. Some promising results have now been obtained and it is likely that a useful method can be proposed in the near future for collaborative test. Meanwhile, some of the pertinent data are summarized in this report.

Experimental

β-Lactoglobulin AB was a lyophilized sample, five times recrystallized, that had been analyzed previously for its amino acid content (2). Potato starch was a commercial product; it was washed thoroughly first with dilute HCl, then with water, alcohol, and ether, and dried in the air.

To accurately weighed samples (2–4 mg) of β-lactoglobulin of known moisture content and 20-fold quantities of starch in 16 × 150 mm test tubes, 2,000-fold quantities (4–8 ml) of 6*N* HCl were added. The tubes were drawn out at the neck, immersed in a Dry Ice bath until the contents were frozen, evacuated to a pressure of less than 1 mm, and sealed under vacuum. After they reached room temperature they were heated for the desired periods of time at 110 ± 1° in an oil bath. In the experiments to be reported here, 24, 72, and 96 hour periods of hydrolysis were used. Because serine and threonine decompose progressively, values for these amino acids are obtained by extrapolation to zero time; because valine and isoleucine are not completely liberated in 24 hours, longer periods of hydrolysis are required.

After hydrolysis, the tubes were cooled and opened. To remove humin the contents of each tube were filtered through a glass fiber filter disk fitted in a coarse, sintered-glass Büchner funnel, and the filtrate was collected in a 50 ml round-bottom, long-neck flask. Five 1 ml portions of hot 1*N* HCl were used as washes for quantitative transfer. The filtrate and washes were concentrated to a sirup in a rotary evaporator, a few ml of water were added, and the evaporation was repeated to remove excess HCl. The residue was dissolved in pH 2.2 buffer (3) containing 5 ml per L of thiodiglycol. It was then either transferred quantitatively to

[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

the 150 cm column of the amino acid analyzer, or it was made to volume (5 ml) and suitable aliquots were applied to both 15 and 150 cm columns. Analyses were run in an automatic analyzer of the type described by Spackman, Stein, and Moore (4). Many of the preceding operations were patterned on the procedures described by Moore and Stein (5), to whom we are indebted for details prior to publication.

Table 1. Amino acid composition of β-lactoglobulin as determined in the absence and presence of starch following hydrolysis in sealed tubes[a]

	g Amino Acid per 100 g Dry Protein	
	β-Lactoglobulin (Reference 2)	β-Lactoglobulin plus Starch
Aspartic acid	11.2	11.3
Threonine	4.94	4.90
Serine	3.64	3.81
Glutamic acid	19.3	19.7
Proline	5.09	5.11
Glycine	1.41	1.36
Alanine	6.98	6.92
Valine	6.03	6.07
Methionine	3.16	2.94
Isoleucine	6.89	7.08
Leucine	15.2	15.5
Tyrosine	3.90	2.92
Phenylalanine	3.55	3.53
Lysine	11.6	11.9
Histidine	1.57	1.65
Arginine	2.79	2.61

[a] The figures are average values for all determinations regardless of time of hydrolysis with the following exceptions: values for threonine and serine are extrapolated values by the method of least squares from analyses of 24, 72, and 96 hour hydrolysates; valine and isoleucine values are averages of analyses from 72 and 96 hour hydrolysates.

Results and Discussion

In Table 1 a comparison is made of the amino acid composition of β-lactoglobulin as determined both in the absence of starch and in its presence. The results of the present analyses are either averaged or extrapolated values from two separate hydrolysates at each time of hydrolysis in the case of the basic amino acids (15 cm column) and at least three different hydrolysates at each time for the other amino acids (150 cm column). The analyses made in the presence of starch have been corrected for small quantities of amino acids found in control hydrolysates of starch alone.

With few exceptions good recoveries of amino acids were obtained. The presence of carbohydrate clearly had no deleterious effect on aspartic acid, threonine, serine, glutamic acid, proline, alanine, valine, isoleucine, leucine, phenylalanine, lysine, and histidine. The small apparent destruction of glycine is thought to be not significant, but the somewhat larger destruction of arginine, though unexpected, may be significant and will bear closer scrutiny.

Cystine values have been omitted because of considerable destruction in both the absence and presence of starch. It is anticipated that in future studies it will be possible to obtain reliable values for cystine (plus cysteine) following performic acid oxidation to cysteic acid by the method of Moore (6). It also will be noted from Table 1 that some destruction of methionine occurred in the present experiments. It may be possible to obtain satisfactory recoveries of this amino acid as well, after similar oxidation to methionine sulfone.

The most serious destruction of an amino acid brought about by the addition of starch to β-lactoglobulin was that of tyrosine, whose recovery was only about 75% of the amount previously found. The lability of tyrosine during acid hydrolysis of protein, especially in the presence of carbohydrate, has been recognized for some time. The expedient of stabilizing tyrosine by adding excess tryptophan before acid hydrolysis was proposed by Lyman, *et al.* (7), and the method was applied to the analysis of various classes of materials used or potentially useful in feeds for poultry and swine. In a few experiments with β-lactoglobulin-starch mixtures to which tryptophan was added, better recoveries of tyrosine were indeed obtained; the amount of additional humin

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formed, however, is substantial and presents special problems, especially when the sealed tube technique is used. Further study of this method is planned. For a number of reasons it seemed desirable to find out whether hydrolysis under reflux, in even more dilute solution than that used in sealed tubes, might not give as good or better results with greater convenience than those obtained in the present series of experiments. Experiments along these lines are in progress and indications are that hydrolysis under reflux may well be the method of choice.

It is recommended that study be continued.

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